

used to isolate a clone or clones containing cDNA inserts. This was accomplished by a modification of the standard "colony screening" method (Sambrook et al, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor N.Y. (1989)). Specifically, twenty 150 mm LB+ampicillin agar plates were spread with 20,000 colony forming units (cfu) of cDNA library and the colonies allowed to grow overnight at 37° C. Colonies were transferred to nylon filters (Hybond™ from Amersham, or equivalent) and duplicates prepared by pressing two filters together essentially as described (Sambrook et al, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor N.Y. (1989)). The "master" plate was then incubated an additional 6-8 hrs to allow the colonies additional growth. The DNA from the bacterial colonies was then bound onto the nylon filters by treating the filters sequentially with denaturing solution (0.5 N NaOH, 1.5 M NaCl) for two minutes, neutralization solution (0.5 M Tris-Cl pH 8.0, 1.5 M NaCl) for two minutes (twice). The bacterial colonies were removed from the filters by washing in a solution of 2X SSC/2% SDS for one minute while rubbing with tissue paper. The filters were air dried and baked under vacuum at 80° C for 1-2 hrs to cross link the DNA to the filters.

Please amend the specification at page 64, line 7 through page ⁶⁶~~65~~, line ¹⁰~~25~~

RP
11/26/08

as follows (Please note that "Expression Analysis" and "RT-PCR" are not being added, rather they are underlined in the specification as filed):

Rapid Amplification of cDNA ends (RACE) was performed following the manufacturer's instructions using a Marathon™ cDNA Amplification Kit (Clontech, Palo Alto, Calif.) as a method for cloning the 5' and 3' ends of candidate genes. cDNA pools were prepared from total RNA by performing first strand synthesis, where a sample of total RNA sample was mixed with a modified oligo (dT) primer, heated to 70° C, cooled on ice and followed by the addition of: 5X first strand buffer, 10 mM dNTP mix, and AMV Reverse Transcriptase (20 U/μl). The reaction mixture was incubated at 42° C for an hour and placed on ice. For second strand synthesis, the following components were added directly to the reaction tube: 5X second strand buffer, 10 mM DNTP mix, sterile water, 20X second strand enzyme cocktail and the reaction tube was incubated at 16° C for 1.5 hours. T4 DNA Polymerase was added to the reaction tube and incubated at 16° C for 45 minutes. The second-strand synthesis was terminated with the addition of an EDTA/Glycogen mix. The sample was subjected to a phenol/chloroform extraction and an ammonium acetate precipitation. The cDNA pools were checked for quality by analyzing on an agarose gel for size distribution. Marathon™ cDNA adapters were then ligated onto the cDNA ends. The specific adapters contained priming sites that allowed for amplification of either 5' or 3' ends, and varied depending on the orientation of the gene specific primer (GSP) that was chosen. An aliquot of the double stranded cDNA was added to the following reagents: 10 μM Marathon™ cDNA adapter, 5X DNA ligation buffer, T4 DNA ligase. The reaction was incubated at 16° C overnight and heat inactivated to terminate the reaction. PCR was performed by the addition of the following to the diluted double stranded cDNA pool: 10X cDNA PCR reaction buffer, 10